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Secondary structure formation during RNA synthesis

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ABSTRACT

We observed the secondary structures that formed in an RNA molecule during its synthesis. Some of the secondary structures seen in nascent chains were observed to form, then to dissociate in favor of an alternative structure, and then to reform, as chain growth continued. The results show that secondary structures in an RNA molecule are in a state of dynamic equilibrium, and that the extension of a sequence by chain growth, or the reduction of a sequence by processing, may result in significant changes in the secondary structures that are present.

INTRODUCTION

We have been studying the formation of secondary structures during RNA-directed RNA synthesis *in vitro* (1), using Qβ replicase (2) and one of its naturally occurring templates, MDV-1 RNA (3). We have determined the sequence of this 221-nucleotide RNA (4, 5) and have used chemical modification to locate the secondary structures that are present in completed molecules (6). In this paper we describe a number of diverse experiments which identify the secondary structures that form in an MDV-1 (-) RNA product strand during its synthesis. Our results show that secondary structures exist in a state of dynamic equilibrium; when a secondary structure is formed it is not necessarily permanent, but rather, it may be replaced by a more stable structure made possible by chain extension. We used band compression patterns in sequencing gels and ribonuclease cleavage patterns of incomplete and full-length molecules to identify the alternate hairpin structures that the RNA could form. The structures that actually do form during the molecule's growth were revealed by the influence of these structures on the rate of chain elongation.

MATERIALS AND METHODS

Nucleotides

[α-^{32}P]GTP, [γ-^{32}P]GTP, and all four 3'-deoxyribonucleoside 5'-[α-^{32}P]triphosphates were obtained from ICN. Unlabeled 3'-deoxyadenosine 5'-triphosphate was purchased from Miles Laboratories, and all other unlabeled ribonucleotides were obtained from P-L Biochemicals.

Qβ Replicase

This RNA-directed RNA polymerase was isolated from Qβ bacteriophage-infected Escherichia coli Q13 by the procedure of Eoyang and August (7), with the hydroxylapatite-chromatography step omitted.

MDV-1 RNA

The synthesis of MDV-1 RNA (8) and the isolation of its complementary (+) and (-) strands by electrophoresis in the presence of Mg^{++} (1) have been described. Since both strands of MDV-1 RNA begin with a guanosine 5'-triphosphate residue (3), [5'-^{32}P]MDV-1 (-) RNA was isolated from synthetic reactions that contained [γ-^{32}P]GTP. A mutant MDV-1 RNA was used for the experiments described in this paper. It differs from wild-type RNA at two positions: there is a uridine in place of a cytidine at nucleotide 86 and an adenosine in place of a guanosine at nucleotide 87. Since the mutant RNA arose from a base substitution that occurred in a single strand of RNA (8), it was, in effect, cloned by selection and is essentially homogeneous. For simplicity, this mutant is referred to as "MDV-1 RNA" throughout this report.

Sequencing Reactions for MDV-1 (-) RNA

Four reactions were prepared. In each, 4.3 µg MDV-1 (+) RNA template and 6.1 µg Qβ replicase were incubated at 37 °C for 8 min in 100 µl 84 mM Tris·HCl (pH 7.4), 12 mM $MgCl_2$, 100 µM ATP, 100 µM CTP, 100 µM GTP, 100 µM UTP, and 500 µM in one of the four 3'-deoxyribonucleoside 5'-[α-^{32}P]triphosphate chain terminators (5). Each reaction mixture was then brought to 1 mg/ml SDS, 10 mM EDTA, and 400 mM NaCl. 10 µg unlabeled yeast RNA (Calbiochem) was added to each as a carrier. The specifically terminated [3'-^{32}P]MDV-1 (-) RNA products in each reaction mixture were isolated by phenol extraction, gel-filtration chromatography, and ethanol precipitation. The RNA products were then melted free of their templates by incubation at 100 °C for 1 min in 50 µl 7 M urea and then quickly chilled to 0 °C. The RNA

from each reaction was analyzed by electrophoresis at 1,000 V on 12 % polyacrylamide slab gels containing 7 M urea and run in 50 mM Tris•borate (pH 8.3), 2 mM EDTA (9).

Ribonuclease Cleavage of Incomplete MDV-1 (-) RNAs

To prepare partially synthesized MDV-1 (-) RNA fragments for use in a structure mapping experiment, 16 µg MDV-1 (+) RNA template and 6 µg Qβ replicase were incubated at 37 °C for 10 min in 125 µl 100 mM Tris•HCl (pH 7.4), 12 mM $MgCl_2$, 32 µM [γ-^{32}P]GTP, 200 µM ATP, 200 µM CTP, 200 µM UTP, and 2 mM 3'-deoxyadenosine 5'-triphosphate. The reaction mixture was then brought to 1 mg/ml SDS, 10 mM EDTA, and 400 mM NaCl. The specifically terminated [5'-^{32}P]MDV-1 (-) RNA products were isolated, melted free of their templates, and separated by electrophoresis on a 12 % polyacrylamide gel, as described above for the products of sequencing reactions. Bands containing the partially synthesized products were located by autoradiography. MDV-1 (-) RNA fragments of 32, 45, 46, 53, 61, and 62 nucleotides in length were excised from the gel. The RNA in each of these bands was recovered by elution in 400 mM NaCl, 3 mM EDTA, 20 mM Tris•HCl (pH 7.5), and was precipitated with ethanol.

Each of the six [5'-^{32}P]MDV-1 (-) RNA fragments was partially digested with ribonuclease T_1 (Calbiochem), as described by Donis-Keller et al. (10). Between 50 and 150 pg of each RNA fragment was dissolved in a 19 µl solution containing all the reaction components listed below except ribonuclease T_1. This solution was incubated at 50 °C for 5 min and then chilled to 0 °C. 1 µl 40 EU/ml (11) ribonuclease T_1 was then added. The final concentration of each component was 7 M urea, 20 mM sodium citrate (pH 5.0), 1 mM EDTA, 250 µg/ml unlabeled yeast RNA, and 2 EU/ml ribonuclease T_1. This solution was incubated at 50 °C for 15 min, melted at 100 °C for 2 min, chilled to 0 °C, and immediately analyzed by electrophoresis on a 12 % polyacrylamide gel containing 7 M urea, as described above. A portion of the specifically terminated [5'-^{32}P]MDV-1 (-) RNA products, that had been saved for later use, was analyzed in parallel with the digestion products to provide size markers.

Direct Observation of MDV-1 (-) RNA Chain Growth

A two-stage reaction was performed. In the first stage, 3 µg MDV-1 (+) RNA template and 15 µg Qβ replicase were incubated at 37 °C for 8 min in 250 µl 84 mM Tris•HCl (pH 7.5), 12 mM $MgCl_2$, 120 µM [α-^{32}P]GTP, and 200 µM ATP (1). Since pyrimidine triphosphates were not present, synthesis of the (-)

strand ceased after the addition of the sixth nucleotide, when the first pyrimidine was required. The reaction mixture was then brought to 2.5 ml, in which the block to further chain elongation was removed by the addition of CTP and UTP, as well as more ATP and unlabeled GTP. Final concentrations in this mixture were 84 mM Tris·HCl (pH 7.5), 12 mM $MgCl_2$, and 2.4 mM (each) ribonucleoside triphosphate. The reaction mixture was then incubated at 4 °C. Since the specific activity of the GTP during this second stage of the reaction was 200-fold lower than during the first stage of the reaction, the label in the MDV-1 (-) RNA product chains was essentially restricted to the four 5'-terminal guanosines. 250-µl samples were drawn from the reaction at 1, 2, 3, 4, and 6 min. Further chain elongation was prevented by bringing each sample to 5 mg/ml SDS and 3 mM EDTA. 40 µg unlabeled yeast RNA was added to each as a carrier. The partially synthesized [5'-^{32}P]MDV-1 (-) RNA products in each sample were isolated by phenol extraction, gel-filtration chromatography, and ethanol precipitation. The RNA products were melted free of their templates by incubation at 100 °C for 2 min in 25 µl 98 % formamide and then quickly chilled to 0 °C. The RNA in each sample was analyzed by electrophoresis at 250 V on a 10 % polyacrylamide gel containing 7 M urea, as described above.

Ribonuclease Cleavage of Full-length MDV-1 (-) RNA

Full-length MDV-1 (-) RNA was partially digested with ribonuclease T_1 by the structure mapping procedure described above for incomplete MDV-1 (-) RNAs. Five reaction mixtures were prepared, each containing 35 ng [5'-^{32}P]MDV-1 (-) RNA in a final volume of 20 µl 7 M urea, 20 mM sodium citrate (pH 5.0), 1 mM EDTA, and 250 µg/ml unlabeled yeast RNA. The final concentration of ribonuclease T_1 in the five reactions was 0.5, 1.58, 5, 15.8, and 50 EU/ml, respectively. The reaction mixtures were incubated at 50 °C for 15 min, chilled to 0 °C, and immediately analyzed by electrophoresis on a 12 % polyacrylamide gel containing 7 M urea, as described above.

RESULTS

The formation of secondary structures in MDV-1 (-) RNA was studied in four experiments. The first two experiments identify stable hairpin structures that can be formed in partially synthesized chains; the third experiment identifies structures that actually form during chain elongation, and provides evidence that structural reorganizations occur as the RNA grows; and the last experiment suggests that structural reorganization also occurs

when chain length is reduced by nucleolytic cleavage.

The results support the sequence of events shown in Fig. 1. By the time MDV-1 (-) RNA is 45 nucleotides long, it contains two hairpin structures, A and B. As the chain grows to 60 nucleotides in length, hairpin B dissociates and is replaced by hairpin T, which forms by the pairing of nucleotides 29-39 with nucleotides 49-60. By the time chain growth reaches 72 nucleotides, another structural rearrangement occurs: hairpin T dissociates in favor of the reformation of hairpin B and the formation of hairpin C at the growing 3' end.

Band Compression Regions in Sequencing Gels

The chain-terminator method of nucleotide sequence analysis (5, 12) depends on the synthesis of four sets of incomplete product strands. Each set contains partially synthesized strands that have the same type of nucleotide at their 3' ends. The different-length strands in each set are separated by electrophoresis in four adjacent lanes of a polyacrylamide gel. The nucleotide sequence is then deduced from the relative positions of the bands that appear in an autoradiograph of the gel. Urea is present in these gels to denature the strands, so that their electrophoretic mobility will be a function of their length. However, strong secondary structures persist in the presence of 7 M urea (5, 13). Consequently, incomplete chains that differ in length by several nucleotides may comigrate. This occurs when the additional nucleotides serve to extend the length of a 3'-terminal hairpin

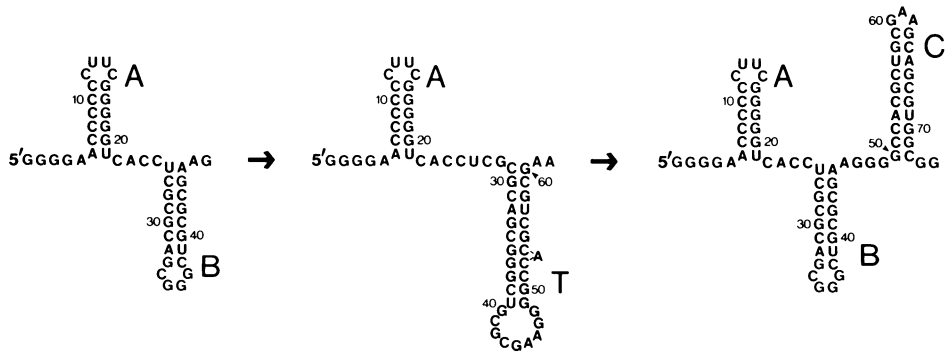

Fig. 1 Structural reorganization during the growth of MDV-1 (-) RNA. Hairpin structures A, B, and C are found in completed chains. Hairpin T is a secondary structure that replaces hairpin B when the growing chain is between 58 and 67 nucleotides long.

stem. The apparent length of the strand is not increased because the sequence folds back on itself. Therefore, bands that represent the 3' side of a hairpin stem have almost identical electrophoretic mobilities, giving rise to a region of band compression in the sequencing gel.

When the nucleotide sequence of an RNA is already known, the band compression regions in a sequencing gel identify the hairpin structures that are present. Fig. 2 shows autoradiographs of sequencing gels that analyze partially synthesized [3'-^{32}P]MDV-1 (-) RNA fragments prepared by the chain-terminator method (5). There are regions of band compression representing the 3' portions of hairpin structures A, B, C, and T (see Fig. 1). The existence of a band compression region between nucleotides 58 and 60 indicates that strands of this length contained hairpin T during

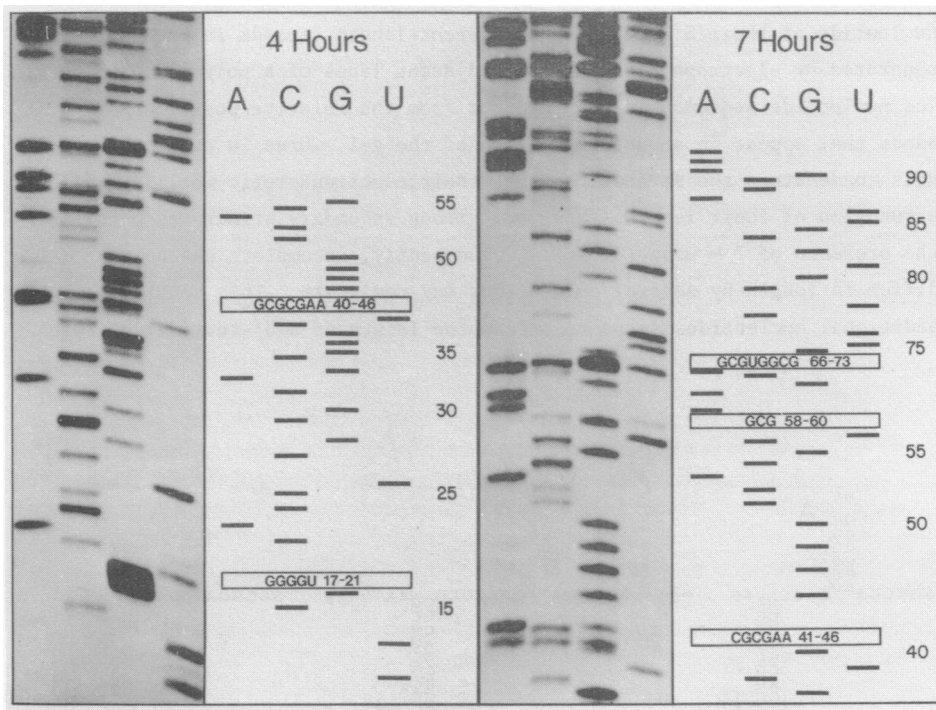

Fig. 2. Sequencing gels of MDV-1 (-) RNA. The duration of electrophoresis is indicated at the top of each key. The nucleotide sequence can be read in the 5' to 3' direction by noting the lane that each band occurs in, beginning at the bottom of each gel. The numbers indicate the length (in nucleotides) of the partially synthesized RNA fragments. Boxes indicate regions of band compression. The sequence within these regions was known from classical sequence analysis of MDV-1 RNA (4).

electrophoresis. Since the evidence also indicates that hairpin B was present in fragments from 41 to 46 nucleotides long, and since hairpins B and T cannot coexist, hairpin B must have dissociated for hairpin T to have been present. Similarly, hairpin T must dissociate for hairpin C to form. It is not possible to tell from these results whether these molecular rearrangements actually occur during chain growth, since the product strands were melted to free them of their templates prior to electrophoresis. The results do indicate, however, that the most stable configuration for partially synthesized chains 58 to 60 nucleotides in length includes hairpin T.

Ribonuclease Cleavage of Partially Synthesized MDV-1 (-) RNAs

Partial digestion with single-strand-specific nucleases is a useful method for locating secondary structures in nucleic acids (14), since nucleotides that occur in double-stranded hairpin stems are resistant to attack. When 5'-labeled RNA is partially digested with a single-strand-specific nuclease and the digestion products are separated by electrophoresis in the presence of urea, the location of each scission site can be deduced from the sizes of the resulting labeled fragments (15). We used this structure mapping technique to identify the hairpin structures that are present in partially synthesized MDV-1 (-) RNAs of known length. Ribonuclease T_1, which specifically cleaves RNA on the 3' side of guanosines that occur in single-stranded regions (16), was used as a probe for the presence of structures.

Partially synthesized [5'-^{32}P]MDV-1 (-) RNAs were isolated from a synthetic reaction containing [γ-^{32}P]GTP to label the 5' ends and unlabeled 3'-deoxyadenosine 5'-triphosphate (as well as ATP) to limit the product strands to those that terminate in an adenosine. These RNAs were separated by electrophoresis, and six fragments, each of a particular length, were isolated from the gel. Each RNA was then partially digested with ribonuclease T_1 in the presence of 7 M urea at 50 °C, as described by Donis-Keller and her colleagues (10). (These conditions mimic those that are present in sequencing gels during electrophoresis, when tertiary structures are eliminated, but strong secondary structures persist (5).) The concentration of ribonuclease T_1 was kept low, in order to limit cleavage to the highly accessible sites that occur in the loops of hairpin structures. Fig. 3 shows an autoradiograph of the gel in which the digestion products were analyzed. Each lane contains the cleavage pattern of one of the six

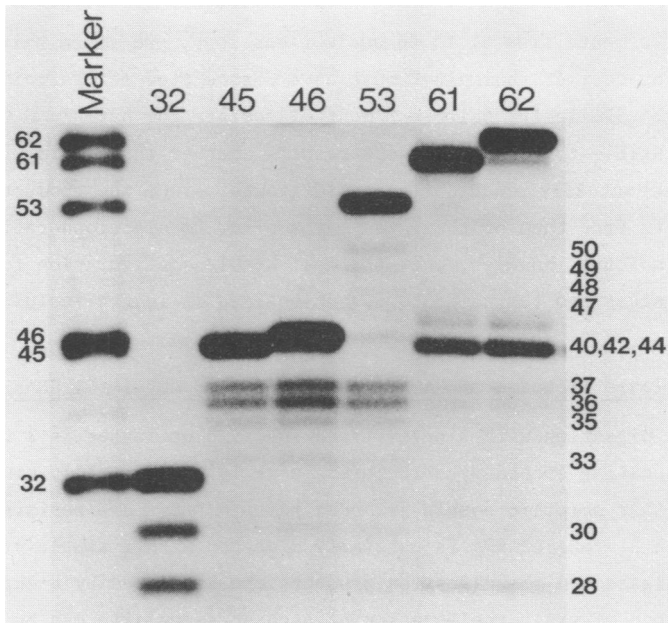

Fig. 3. Ribonuclease T_1 cleavage patterns of partially synthesized [5'-^{32}P]MDV-1 (-) RNAs. The original length (in nucleotides) of each 3'-deoxyadenosine-terminated fragment is indicated across the top of the autoradiograph. The marker lane contains a sample of the mixture from which these specific-length fragments were isolated. Numbers along the sides of the autoradiograph identify the length of the RNA in each band. Numbers on the left refer to undigested fragments, and numbers on the right refer to their ribonuclease T_1 cleavage products.

RNAs. Partially synthesized chains of length 45, 46, or 53 nucleotides were primarily cleaved at nucleotides 36 and 37, which occur in the loop of hairpin B (see Fig. 1). However, partially synthesized chains of length 61 or 62 nucleotides were primarily cleaved at nucleotides 40, 42, and 44, which occur in the loop of hairpin T. These results indicate that the most stable configuration for chains of 61 or 62 nucleotides in length includes hairpin T.

Overabundant Elongation Intermediates

We have shown that RNA chain elongation occurs at a highly variable rate (1). This was observed by sampling reactions in which MDV-1 (-) RNA was being synthesized, and then analyzing the different partially synthesized RNAs by electrophoresis. A few of these elongation intermediates were much

more abundant than others. Sequence analysis of these incomplete chains indicated that their overabundance is caused by a marked decrease in the rate of elongation at sequence positions where the growing end of the RNA can form a hairpin structure

Although band compression regions in sequencing gels and ribonuclease cleavage patterns identify the most stable structures that can occur in a partially synthesized RNA, they do not show that these structures actually form during growth, since the RNAs must be melted during their isolation. The analysis of prominent elongation intermediates, however, does indicate which structures actually form, since the relative abundance of the intermediates is not altered by isolation or electrophoresis. Fig. 4 shows the results of an experiment in which the growth of [5'-^{32}P]MDV-1 (-) RNA was observed by analyzing the elongation intermediates that were present at various times after synthesis was initiated. Although the average chain length of the product RNA increased with time, most of the partially synthesized chains occurred in a few bands of discrete length. The length of the RNA in these bands was determined by nucleotide sequence analysis (1). The presence of prominent bands containing RNA of 21-23, 43-44, and 71-72 nucleotides indicates that hairpins A, B, and C, respectively (see Fig. 1), actually form at the growing end of MDV-1 (-) RNA in the course of chain elongation. The presence of a band containing RNA of 60-61 nucleotides indicates that hairpin T also forms during chain growth. Since hairpin T cannot coexist on the same strand with hairpins B and C, the results suggest that these molecules undergo structural rearrangements.

Structural Reorganization during Partial Ribonuclease Digestion

We used the ribonuclease cleavage technique described above to locate secondary structures in full-length MDV-1 (-) RNA. Our results indicate that structural reorganizations may also occur when a sequence is reduced in length by nucleolytic cleavage.

[5'-^{32}P]MDV-1 (-) RNA was isolated from a synthetic reaction containing [γ-^{32}P]GTP and then partially digested with ribonuclease T_1. Fig. 5 shows an autoradiograph of the gel in which the digestion products were analyzed. A second lane containing 3'-deoxyguanosine-terminated MDV-1 (-) RNA products was used to mark the location of bands corresponding to each guanosine in the sequence. Although band compression makes it difficult to resolve some bands (5, 13), many bands that occur in the terminated lane are clearly missing from the ribonuclease-generated lane, indicating the presence of secondary

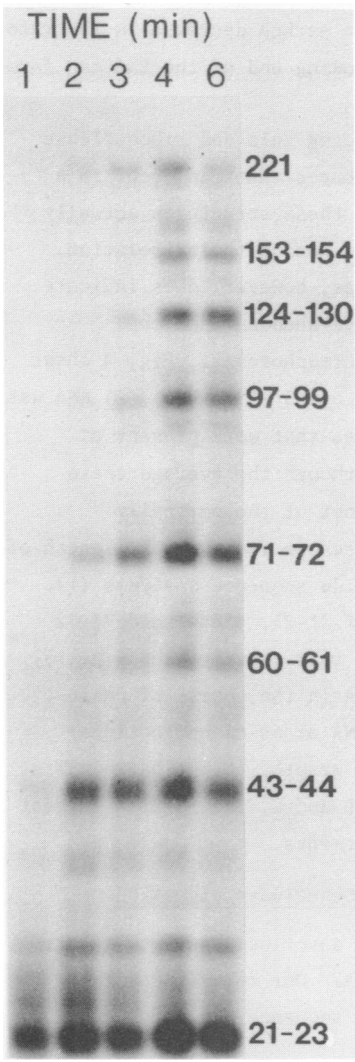

Fig. 4. Electrophoretic analysis of partially synthesized [5'-^{32}P]MDV-1 (-) RNAs present in a reaction at various times after the initiation of synthesis. Numbers indicate the length (in nucleotides) of the RNA in each band. A secondary structure occurs at the 3' end of each of these elongation intermediates. Although the size of each intermediate is given as a range, they may actually be homogeneous. The uncertainty in the length arises from the classical nucleotide sequencing procedure we used (1).

structures. For example, bands that would indicate cleavage at nucleotides 16-20, 50, 55, 63, and 66-73 are missing. In addition, bands 42-49 are weakly represented.

The cleavage pattern in Fig. 5 was interpreted by examining the expected secondary structures shown in Fig. 6. Single-stranded guanosines in the loops of hairpin structures, such as nucleotides 35-37, 60, and 90, are very accessible to ribonuclease T_1. Single-stranded guanosines that occur between

the bases of hairpin stems, such as nucleotides 47, 48, and 49, are partially protected from the nuclease, which cleaves them less frequently, giving rise to weak bands. Guanosines located in double-stranded hairpin stems, such as nucleotides 16-20, cannot be cleaved unless the hairpin is first cleaved at a guanosine located in the hairpin loop. When loop cleavage does occur, as in the loop containing nucleotides 35-37, the hairpin stem is free to melt apart, and the guanosines that had been protected by the hairpin stem can be cleaved. These secondary cleavages account for the presence of bands representing nucleotides 28, 30, and 33, and also 80, 82, and 84. These bands arise from cleavages at guanosines that originally occurred on the 5' side of hairpin stems. Although secondary cleavages also occur at guanosines that were present on the 3' side of hairpin stems, such as nucleotides 63, 66, 68, 70, and 71, they do not give rise to bands because the resulting fragments are not labeled.

This interpretation does not explain the absence of the bands representing the guanosines at positions 50 and 55, which occur on the 5' side of hairpin C. Cleavage at nucleotide 60 in the hairpin loop should have led to secondary cleavages at nucleotides 50 and 55. The absence of these bands indicates that after the cleavage at nucleotide 60, the 60-nucleotide-long fragment underwent a rapid structural reorganization, in which hairpin B dissociated and hairpin T was formed. Although the newly formed hairpin T is subject to cleavage in its loop (seen as the weak band representing nucleotides 42 and 44), nucleotides 50 and 55 are located on the 3' side of hairpin T, and their subsequent cleavage would not yield labeled fragments. The complete absence of these bands indicates that secondary structures can reorganize into a more stable configuration when the primary sequence is reduced by nucleolytic cleavage.

DISCUSSION

Band compression regions in sequencing gels (Fig. 2) and ribonuclease cleavage patterns of partially synthesized strands (Fig. 3) identify the most stable configurations for partially synthesized strands of different length. These results show that the most stable configuration for MDV-1 (-) RNA strands between 41 and 57 nucleotides in length includes hairpin B, while the most stable configuration for strands between 58 and 67 nucleotides includes hairpin T. The most stable configuration for strands longer than 67 nucleotides includes hairpin C.

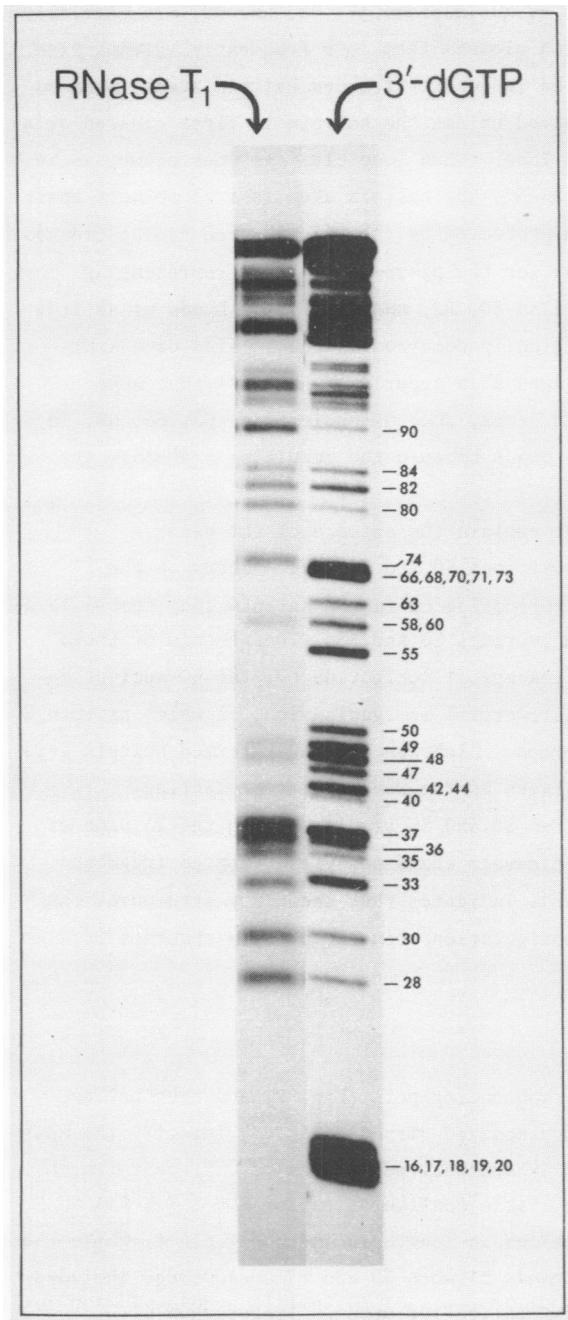

Fig. 5. Ribonuclease T_1 cleavage pattern of full-length [5'-^{32}P]MDV-1 (-) RNA. The partial digest shown above was prepared in a reaction containing 5 EU/ml ribonuclease T_1. Other ribonuclease T_1 concentrations, over the range 0.5 to 50 EU/ml, were tried (results not shown). Although there was almost no digestion of the RNA at 0.5 EU/ml and almost complete digestion into small fragments at 50 EU/ml, bands that were missing at 5 EU/ml did not appear at any other concentration of the ribonuclease, indicating that the absence of bands was not due to the kinetics of digestion, but rather, to the inherent susceptibility of the RNA to cleavage.

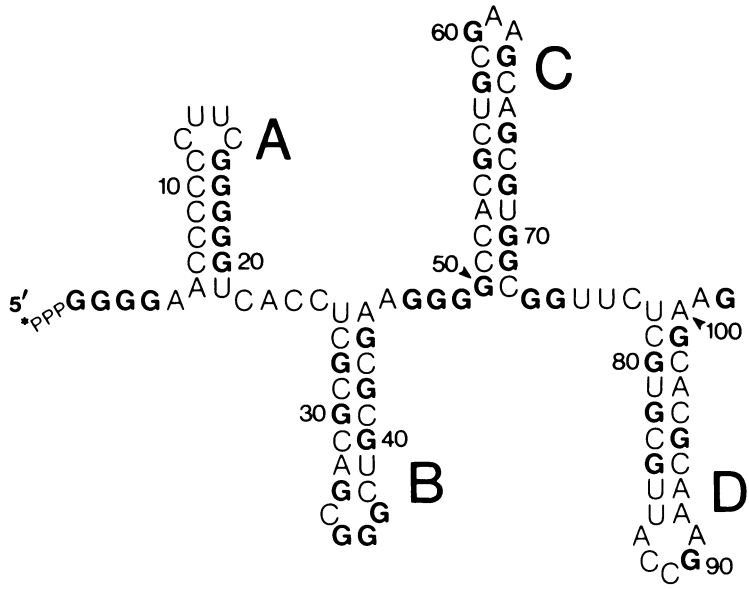

Fig. 6. Nucleotide sequence of the 5' end of MDV-1 (-) RNA showing the hairpin structures that are present in completed chains. The guanosine residues are emphasized to aid in the interpretation of the structure mapping results. An asterisk identifies the labeled phosphate.

Hairpins B and T cannot coexist on the same strand, since they share regions of the MDV-1 (-) RNA sequence. Similarly, hairpins T and C cannot coexist. Yet, the results shown in Fig. 4 indicate that hairpins B, T, and C all occur during chain elongation. These observations can be interpreted in two ways: either structural reorganizations occur during chain growth, leading to the replacement of less stable configurations by more stable configurations (as shown in Fig. 1), or alternate folding pathways are followed during chain growth, in which some strands form hairpin T, while other strands form hairpins B and C.

Structural Reorganizations

We favor the interpretation that structural rearrangements occur. Both hairpin B and hairpin T are present during chain growth, since there are prominent elongation intermediates of length 43-44 nucleotides and 60-61 nucleotides (see Fig. 4). Yet, they cannot coexist on the same molecule. These results suggest that hairpin B is present during chain growth, prior to the formation of hairpin T, but once MDV-1 (-) RNA grows sufficiently long

for hairpin T to be potentially more stable than hairpin B (at approximately 58 nucleotides), a structural rearrangement occurs yielding hairpin T. Similarly, additional chain growth leads to the dissociation of hairpin T in favor of the formation of hairpin C and the reformation of hairpin B, which together contribute more to the stability of the RNA than hairpin T alone. Thus, as an RNA chain is synthesized, secondary structures are formed, and when the addition of nucleotides to the growing end makes it possible to form a more stable configuration, structural reorganization occurs.

The ribonuclease cleavage results shown in Fig. 5 suggest that deletions in RNA molecules can also induce structural rearrangements. After scission of MDV-1 (-) RNA at nucleotide 60 causes the destruction of hairpin C, the presence of hairpin B in the cleavage product is a less stable configuration than one that includes hairpin T. The evidence suggests that hairpin B dissociates and hairpin T takes its place before any significant cleavage occurs at nucleotide 50 or 55. These results are consistent with the view that secondary structures exist in a state of dynamic equilibrium, in which less stable configurations are replaced by more stable configurations when the primary sequence is altered by growth or nucleolytic cleavage.

Alternate Folding Pathways

Structural reorganizations need not occur for mutually exclusive hairpins to be observed during chain growth. Although hairpins B and T cannot coexist on the same strand, they can occur on different strands. Some strands might not form hairpin B, but instead would form hairpin T when they had grown sufficiently long. Similarly, when strands that contain hairpin B grow longer, they could form hairpin C without ever forming hairpin T.

These alternate folding pathways can only occur if less stable molecular configurations persist during chain growth. For example, if some chains form hairpin T without ever having formed hairpin B, they would have gone through a stage of growth in which they were less stable than if they had formed hairpin B. This can occur when the rate of polymerization is so fast that chains are extended to a point where hairpin T can form before hairpin B forms. However, polymerization is slow compared with the rate of structure formation, since the progress of the replicase is repeatedly delayed by newly formed structures (1). We therefore think that alternate folding pathways is not the best explanation for the occurrence of hairpins B and T. Instead, we favor the interpretation that a single folding pathway is made possible by structural rearrangements that yield the most stable configuration at every

stage of growth.

Biological Significance

If the configuration of an RNA is generally capable of change and may comprise a different set of secondary structures at different stages of chain growth, it should prove fruitful to explore the biological significance of this flexibility. Since secondary structures often play important functional roles during replication, partially synthesized strands may, by virtue of their changing configuration, function in a quite different manner than completed strands. For example, alternate configurations may affect the binding of polymerases and ribosomal subunits to nascent strands, they may prevent collapse of the product strand upon the template during replication, and they may serve as regulatory signals controlling the termination of transcription. Similarly, nucleolytic cleavage and splicing of primary gene transcripts produces shorter sequences that may undergo structural reorganizations. These processed transcripts may, by virtue of a new molecular configuration, be rendered biologically active. Thus, an awareness of the dynamic aspects of secondary structure formation may lead to an increased understanding of replication and the regulation of gene expression.

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REFERENCES

1. Mills, D. R., Dobkin, C. and Kramer, F. R. (1978) Cell 15, 541-550
2. Haruna, I. and Spiegelman, S. (1965) Proc. Natl. Acad. Sci. USA 54, 579-587
3. Kacian, D. L., Mills, D. R., Kramer, F. R. and Spiegelman, S. (1972) Proc. Natl. Acad. Sci. USA 69, 3038-3042
4. Mills, D. R., Kramer, F. R. and Spiegelman, S. (1973) Science 180, 916-927
5. Kramer, F. R. and Mills, D. R. (1978) Proc. Natl. Acad. Sci. USA 75, 5334-5338
6. Mills, D. R., Kramer, F. R., Dobkin, C., Nishihara, T. and Cole, P. E. (1980) Biochemistry 19, 228-236
7. Eoyang, L. and August, J. T. (1971) in Procedures in Nucleic Acid Research, Cantoni, G. L. and Davies, D. R. Eds., Vol. 2, pp. 829-839, Harper and Row, New York

8. Kramer, F. R., Mills, D. R., Cole, P. E., Nishihara, T. and Spiegelman, S. (1974) J. Mol. Biol. 89, 719-736
9. Maniatis, T., Jeffrey, A., and van deSande, H. (1975) Biochemistry 14, 3787-3794
10. Donis-Keller, H., Maxam, A. M. and Gilbert, W. (1977) Nucleic Acids Res. 4, 2527-2538
11. Takahashi, K. (1961) J. Biochem. 49, 1-8
12. Sanger, F., Nicklen, S. and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467
13. Mills, D. R. and Kramer, F. R. (1979) Proc. Natl. Acad. Sci. USA 76, 2232-2235
14. Kahn, M. S. N. and Maden, B. E. H. (1976) FEBS Lett. 72, 105-109
15. Wurst, R. M., Vournakis, J. N. and Maxam, A. M. (1978) Biochemistry 17, 4493-4499
16. Sato-Asano, K. (1959) J. Biochem. 46, 31-37